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(54) **A process for the production of refined fish oil concentrate.**

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Description

The present invention concerns a process for producing refined fish oil concentrate. In the refining process, cholesterol and useful by products such as urea adducts of fatty acid compounds are produced, in addition to higher unsaturated fatty acids.

It is known that waste products from the fish refining industry contain usable products, among others fatty acids, cholesterol, proteins and enzymes. These are either fat-soluble or water-soluble. Such waste products are normally referred to as fish entrails.

Through the processing of fish entrails, the water-soluble portion containing proteins and enzymes may be separated from the fat-soluble portion. The present invention concerns the fat-soluble portion of the waste products, but it can also use other refined fish oils such as occur for instance in the fish product industry. In the following these basic raw materials will be called « fish oil product ».

It is known that certain essential fatty acids in fish oil have a medicinal effect and are useful in the prevention and cure of thrombotic illnesses, for instance ischemic heart disease. In addition, these compounds lower the cholesterol level in the blood.

Among the above-mentioned fatty acids, the following may be specified as suitable for the medicinal purposes referred to: eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Both fatty acids are ω 3-fatty acids of the C-20 and C-22 acids. Their nomenclature according to the IUPAC system is :

for the eicosapentaensoic acid (EPA) :

cis — 5, 8, 11, 14, 17 — eicosapentaenoic acid ;

and for the docosahexaenoic acid (DHA) :

cis — 4, 7, 10, 13, 16, 19 — docosahexaenoic acid ;

which are sometimes abbreviated to :

eicosapentaenoic acid 20 : 5 ω3, and

docosahexaenoic acid 22 : 6 ω3

where 20 and 22 indicate the number of C-atoms respectively in the molecule of the fatty acid, 5 and 6 the number of unsaturated bondings, and ω3 that the last unsaturated bonding is positioned at a distance of 3 carbon atoms from the ω-position.

In the following we will be using the designations EPA and 20 : 5 ω3 for the eicosapentaenoic acid and DHA and 22 : 6 ω3 for the docosahexaenoic acid.

The fat soluble portion of cod entrails, for instance, usually contains 10-25 % of the fatty acid compounds EPA and DHA, sometimes referred to herein as the essential fatty acid compounds, as well as 2-4 % cholesterol. The remainder is mainly fatty acid compounds with lower unsaturation, such as pure fatty acids or their glycerides.

The purpose of the invention is to treat fish oil product so as to separate the fatty acid compounds EPA and DHA from cholesterol and other fatty acid compounds, and thereby from a concentrate containing high concentrations of EPA and DHA.

It has long been known that the easiest way to separate fatty acids is by means of extraction or distillation when they appear in the form of esters, for instance as methyl or ethyl esters. Before treatment, fatty waste products from the fish industry must have been subjected to trans-esterification and esterification, for instance with methanol for production of fatty acid methyl esters.

This basic material is well suited for further separation of EPA and DHA and cholesterol from the remaining less important fatty acid compounds.

In US-A-4,145,446 a method is described for precipitation of fatty acid compounds in raw material by means of urea. The purpose is to obtain a product containing proteins and fats, suitable for fodder. The production of the urea adduct is brought about by providing a solution of melted urea at 60-140 °C to which is added melted fatty acid or a mixture of fats heated to a temperature of 35-105° so that the ratio fat/fatty acids to urea will be from 40/60 to 60/40 units by weight.

Another patent, SU-A-950,393 describes a method for production of cholesterol from, for instance, fish waste products by hydrolysing the fatty acid compounds and converting them to soaps. These are then subjected to an extraction of trichlorethylene at room temperature, whereby the cholesterol is combined with the trichlorethylene and this compound is then subject to further separation.

GB-A-1,240 513 also concerns a separation technique by means of urea where the raw material consists of pure methyl and ethyl esters of the C_{16}-C_{18} fatty acids. Urea precipitation occurs in a neutral environment with a surplus of the relevant alcohol. The purpose is to be able to obtain a stronger concentration of γ-linolenic acid. The above-mentioned fatty acid esters do not contain any higher fatty acids other than C-18 in the form of stearic acid, oleic acid, linoleic acid and linolenic acid, which after the urea precipitation and separation of the urea adduct from the rest of the material has obtained a higher content of γ-linolenic acid by means of chromotography.

The higher unsaturated fatty acid compounds 20 : 5 ω3 and 22 : 6 ω3 may be concentrated according to a method described in Japanese Patent Publication No. 59-071396 where the fatty acid compounds mentioned are extracted by means of polar solvents, such as acetone, methyl ethyl ketone, methanol, ethanol, and similar solvents, whereby a soluble and an insoluble extract are formed. Thereafter the extract is further processed to obtain essential fatty acid compounds.

According to the present invention it is now possible — in a remarkably simple manner — to

optimize the procedure to increase the concentration of ω3-fatty acid compounds and cholesterol. This is based on a fractionated precipitation of the less interesting fatty acid compounds with urea, since urea tends to form an adduct with fatty acids which do not belong to the ω3 type, wherens fatty acids of ω3 type do not. Nor does cholesterol form an adduct with urea. The procedure used previously was to isolate the fatty acid compounds before these were esterified separately, and then they were subjected to a fractionated precipitation with urea. This procedure is unnecessary with our invention.

Thus, the present invention provides a process for the production of a refined fish oil concentrate containing at least 20 % eicosapentaenoic acid (EPA) and at least 35 % docosehexaensoic (DHA) by weight, the remainder of the concentrate including other unsaturated fatty acid compounds, and the fatty acid compounds of the concentrate being mainly present as alkyl esters of lower alcohols, which comprises the steps of :

(a) esterifying and/or trans-esterifying the fat/fatty acid fraction of fish oil product at room temperature with a lower alcohol in an alkaline environment containing amounts of base sufficient only to catalyze the esterification and/or trans-esterification reaction ;

(b) heating the resulting alkyl ester product with an excess of urea in an alkanol to from 55-90 °C;

(c) cooling the product of step (b) to 0 °C to precipitate urea fatty acid alkyl ester adduct, and thereafter separating off said adduct to leave a solution mainly containing ω3-fatty acid esters and an unsaponifiable portion ;

(d) separating from the solution remaining from step (c) the ω3-fatty acid alkyl esters and the unsaponifiable portion, preferably by extracting with a solvent ;

(e) removing any solvent from the mixture obtained in step (d) ; and

(f) cooling the concentrate obtained in step (e) to crystallize out cholesterol and to precipitate out other undefined unsaponifiable compounds, thereby to leave a refined fish oil concentrate.

A one special feature of this process is that fatty acid compounds are not separated prior to precipitation of the urea adduct, but instead precipitate from the same non-uniform mixture of components like they are found in the basic raw material.

Another special feature is that the precipitation of the urea adduct takes place in an alkaline environment, and in such a way that the alkaline environment is created through applying the base only in catalytic quantities such as a catalytic agent for the trans-esterification of glycerides to methyl esters and not as a means of saponification of the fatty acids.

A third special feature is that the esterification and/or trans-esterification takes place at room temperature.

A result of esterification or trans-esterification at low temperature and in an environment with low alkalinity is that isomerization of the double bonds is avoided, which results in a more uniform product with no toxic effect. At the same time transconfigurations are avoided. The remaining solution is thereafter extracted by means of a non-polar solvent, for example hexane, whereby the ω3-fatty acids as well as cholesterol will be found in the non-polar phase.

Thereafter the non-polar phase is treated to remove the solvent, as by being subjected to evaporation of the solvent under moderate conditions, for instance by means of vacuum distillation. The remaining ω3-concentrate now contains all the cholesterol, and it becomes apparent that the cholesterol does not dissolve easily in this concentrate and will crystallize on cooling. The ω3-concentrate which is left will contain 20-30 per cent EPA by weight and 35-50 % DHA by weight. The remainder of the concentrate consists mainly of non-essential fatty acid compounds which are not important for our purpose.

For a better understanding of the invention, we refer to the block diagram in Fig. 1 which shows diagrammatically one way of carrying out the invention and where each block represents a step in the process and is marked with a reference number. The flow of the material to and from each block and between the blocks is marked by solid and dotted lines. In addition, each material is characterized by a letter.

The basic material is fat and/or fatty acids from fish and especially fat and/or fatty acids obtained from the fish processing industry in connection with ensilage and or auto-catalysis processes, but the process may also be used with other forms of marine fat. This fatty raw material is called fish oil product in the claims.

Such fat/fatty acids have a high content of saturated, unsaturated and polyunsaturated fatty acids with a chain length C 18, C 20 and C 22 as well as a certain amount of cholesterol, vitamins and other fat soluble products which are undefinable, usually characterized as unsaponifiable, as well as fatty acid compounds with shorter chain lengths.

Box 1.

Fat/fatty acids (A) from fish with a content of i. a. cholesterol, also called the fish oil product, is placed in a container for trans-esterification with an alcohol with a low boiling point (B), for instance methanol or ethanol, preferably methanol, and a catalytic agent as well as auxiliary compounds (C) to obtain a faster esterification and trans-esterification in order to prevent oxidization and dis-coloration. Potassium hydroxide may be used as a catalytic agent, and in order to prevent oxidization, especially when heavy metals such as chromium, iron, cobalt, nickel and copper are present, small amounts of the sodium salt of ethylenediaminetetra-acetic acid ($EDTA\text{-}Na_3$) may be added. The esterification and trans-esterifi-

cation take place under moderate conditions and stirring at about 20 °C for some hours. The formation of alkyl esters is nearly complete when the ester product has changed its appearance from opalescent to clear. The clear solution (D1) therefore contains alkyl esters of the fatty acids, glycerol, alkanol, as well as some water from the esterification of the free fatty acids.

Box 2.

The clear solution is then heated to a temperature of 55-90°, preferably 60-80°, most preferably 65-68 °C, whereafter a fixed amount of urea (E) and alkanol (B) is added and stirred in until everything is completely dissolved. The amount of urea depends on the composition of the fatty acids so that if the raw material (A) contains 6-8 % EPA by weight, urea is added in the ratio 3 parts urea by weight to 1 part alkyl ester. In order to ensure that the components are completely soluble, 9 parts alkanol by weight is added.

When everything is dissolved, the solution is slowly cooled to approx. 20 °C. An adduct of urea fatty ester (F) is crystallized and then removed by means of for instance decanting and filtration, whereafter the filter mass is cooled to 0 °C in order to crystallize a larger portion of the adduct (F). The adduct is then separated by a known method so that the remaining filter mass (G) contains the essential fatty acid fractions and the unsaponifiable fractions.

Box 3.

The slightly alkaline filter mass (G) is saturated with hexane or other non-polar solvent, preferably hexane, and is extracted by means of this solvent through a known technique, as, for instance, by a continuing fluid-to-fluid counter current process, whereafter a further quantity of adduct of urea fatty ester may be crystallized. By means of this extraction two fluids are formed, consisting of hexane (I) and a residue (K).

Box 4.

The hexane extract (I), which contains the alkyl-fatty esters of the polyunsaturated fatty acids 18 : 4 ω3, 20 : 5 ω3- and 22 : 6 ω3 as well as cholesterol as the most important components, is washed in dilute hydrochloric acid (L) in order to neutralize possible potassium soaps of the essential polyunsaturated fatty acids in the hexane extract. The washing water is decanted.

Hexane (H) is thereafter removed by evaporation of the extract (I) so that a concentrate is produced which is free from solvents (N) and which contains the compounds that are essential for the invention, the fatty acid compounds EPA and DHA as well as cholesterol.

The dehydrated extract normally contains 20-30 % EPA, 35-45 % DHA, 10-20 % other polyunsaturated fatty acids, as well as 5-15 % cholesterol and undefined compounds, all by weight, but the exact composition referred to will depend on the type of fish used, the time of year the fish is caught, and the type and condition of the raw material.

Box 5.

The concentrate of alkyl fatty acid ester (N) is thereafter cooled to approx. minus 25 °C, whereafter cholesterol (O) is crystallized. This is centrifuged and/or filtered.

Further impurities which are present in the concentrate (N) may be removed by cooling the mixture to a temperature of lower than minus 25 °C, whereafter certain undefined compounds are precipitated and filtered by means of a known method. The remaining ω3-concentrate (P) thus contains 20-35% EPA, 35-50 % DHA and 15-25 % of other polyunsaturated ω3-fatty acid compounds (all by weight) as well as unsaturated fatty acid compounds which are not essential for the invention.

Product (p) which contains the alkyl esters of the corresponding ω3-fatty acids may be utilized as it is or if desired the concentration of EPA and DHA may be increased.

Since the product contains only small amounts of other fatty acids with the same chain length as EPA and DHA, it is well suited for separation of the essential fatty acids, EPA and DHA, by means of supercritical fluid extraction.

Another method for increasing the concentration is by means of preparative liquid chromatography by which method a more than 90 % purity of the essential fatty acids is obtained.

Box 6.

The alkaline residue (K) is acidified by means of concentrated hydrochloric acid (L) to a pH = 2, whereafter a hexane fraction (R) is precipitated in an upper layer which is separated. One may also subject the acid solution (S) to further extraction by means of hexane if this should be necessary, whereafter the hexane extracts are gathered. The hexane fractions (R) contain free fatty acids as well as some of their alkyl esters and fairly high percentages of EPA and DHA, but also a fairly substantial portion of C 18-, C 20- and C 22-fatty acids with lower unsaturation. This acid solution contains water, alcohol, alkanol, glycerol, urea and other products which may be retrieved by a separate process which is not described here.

Box 7.

The fatty acid components of the hexane fraction are increased by evaporating hexane (H) in a separate piece of equipment.

Box 8.

The remaining solution is esterified using lower alkanols, for instance methanol or ethanol by means of an appropriate catalytic agent (V) which

for instance may be dehydrated hydrochloric acid, acetic acid chloride or boron-trichloride.

The resultant alkyl ester (D2) from the fatty acid components (T) from box 7 may be processed in various ways, for instance returned to box 2 for urea precipitation of the less unsaturated fatty acids.

Example

To 50 kg fish oil product (A) from cod entrails (containing 8 % EPA, 11 % DHA, and 2.3 % cholesterol all by weight) 400 l methanol (B) and 10 g EDTA Na3 (C) were added in a reactor. Potassium hydroxide (C) dissolved in methanol was added for neutralisation of free fatty acids in a quantity corresponding to a colour reaction of pH 12 on a moist pH-paper. Thereafter 50 l methanol (B) were added.

The whole mixture was subjected to stirring for 15 hours at 20 °C in order to bring about a trans-esterification of the glycerides to methyl esters and esterification of the free fatty acids to methyl esters.

When the trans-esterification was complete, the temperature was increased to 65-68 °C and 140 kg urea (E), as well as a fixed amount of methanol (B) were stirred in while being heated until everything seemed to be dissolved. Then the solution was slowly cooled to room temperature (approx. 20 °C), whereafter a urea adduct of fatty acid was precipitated (F). It contained the major portion of the saturated and lower unsaturated fatty acid methyl esters.

The urea adduct was separated from the solution by decanting and filtering according to an ordinary, known technique. Result: 100.1 kg urea adduct (F).

Thereafter the solution was cooled to 0-4 °C, whereby an additional 5.1 kg urea adduct (F) could be filtrated from the solution.

This filtrate (G) contained ω3-polyunsaturated fatty acid methyl esters, cholesterol and a residue of unwanted fatty acid fractions with lower unsaturated C 18, C 20 and C 22 fatty acid methyl esters. To this filtrate we added hexane for saturation, whereby a further amount of urea adduct (22 kg) could be separated. This hexane-saturated solution was extracted in a counter-current with hexane so that the hexane extract (I) finally made up approx. 300 l. The remaining unextracted solution is called (K). The hexane extract was thereafter evaporated. The yield of ω3-fatty acid methyl ester concentrate : 10.2 kilos.

The concentrate (N) which contained 23 % EPA, 41 % DHA and 8 % cholesterol, all by weight, was thereafter cooled to minus 25 °C, whereby pure cholesterol (O) crystallized and was removed by means of centrifuging during which time the residue in the centrifuge was washed with hexane with a lower temperature in order to remove the fatty acid methyl esters from the cholesterol crystals. Yield : 760 g pure cholesterol. The concentrated filtrate (P) contained 25 % EPA-methylester, 43 % DHA-methylester by weight based on the fatty acid portion, and traces of cholesterol.

The above-mentioned extracted residue (K) was cleansed with a solution of concentrated hydrochloric acid, whereby one hexane phase could be filtered of f. Additional hexane was added to the batch, stirred and then precipitated. The hexane fractions were put together and the hexane evaporated. To 7.7 kilos of the remaining fatty acid and the methyl fatty acid fraction, 15 liter 2 % methanolic solution of boron trichloride were added.

Yield : 6 kilos methyl fatty acid esters, containing 13 % EPA-methylester, 17 % DHA-methylester and approximately 2 % cholesterol, all by weight.

The methyl fatty acid concentrate, containing methanol, was returned to the process for treatment with urea.

With this invention it has been possible to produce a very pure ω3-concentrate of fatty acid alkyle esters, where the essential anti-thrombotic fatty acid components eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are present in a high concentration.

Further, by means of the procedure invented, it has been possible in a simple manner to separate very pure and crystalline cholesterol. An additional product is a urea adduct of fatty acid components, but this is not of current interest.

Another advantage with the invention is that it is possible to produce a urea adduct without following the cumbersome procedure of first producing the fatty acids, esterify these with alkanol and then separating them by means of the fractionated urea precipitation.

By following the procedure invented, it is also possible to avoid the formation of emulsions in the phases, and the phase separation is thereby facilitated during later extraction stages.

Claims

1. Process for the production of a refined fish oil concentrate containing at least 20 % eicosapentaenoic acid (EPA) and at least 35 % docosehexaensoic acid (DHA) by weight, the remainder of the concentrate including other unsaturated fatty acid compounds, and the fatty acid compounds of the concentrate being mainly present as alkyl esters of lower alcohols, which process comprises the steps of :

(a) esterifying and/or trans-esterifying the fat/fatty acid fraction of fish oil product at room temperature with a lower alcohol in an alkaline environment containing amounts of a base sufficient only to catalyze the esterification and/or trans-esterification reaction ;

(b) heating the resulting alkyl ester product with an excess of urea in an alkanol to from 55-90 °C ;

(c) cooling the product of step (b) to 0 °C to precipitate urea fatty acid alkyl ester adduct and thereafter separating off said adduct to leave a solution mainly containing 3-fatty acid esters and an unsaponifiable portion ;

(d) separating from the solution remaining from step (c) the 3-fatty acid alkyl esters and the unsaponifiable portion, preferably by extracting with a solvent ;

(e) removing any solvent from the mixture obtained in step (d) ; and

(f) cooling the concentrate obtained in step (e) to crystallize out cholesterol and to precipitate out other undefined unsaponifiable compounds, thereby to leave a refined fish oil concentrate.

2. Process according to Claim 1, wherein the concentrate obtained in step (f) is further treated in order to increase the concentration of EPA and DHA therein.

3. Process according to either one of Claims 1 and 2, wherein, in step (b), the fatty acid alkyl esters are treated with urea at a temperature of from 60-80 °C, whereby the urea fatty acid alkyl ester adduct in the main does not contain 3-fatty acid compounds and unsaponifiable compounds.

4. Process according to any preceding claim wherein, in step (d), the separation is effected by extraction with hexane.

5. Process according to Claim 4, wherein, prior to step (e) the hexane extract is cleansed with a dilute acid, preferably hydrochloric acid.

6. Process according to any preceding claim, wherein step (f) is carried out by first cooling the 3-fatty acid alkyl ester concentrate to a temperature not lower than —25 °C, whereby cholesterol is crystallized out, and thereafter to —50 °C whereby the remaining portion of the unsaponifiable compounds precipitates.

7. Process according to any preceding claim, wherein methanol is used in step (a), whereby the fatty acid compounds of the concentrate obtained are mainly present as methyl esters.

8. Process according to any preceding claim, wherein the refined fish oil concentrate obtained in step (f) contains 20-30 % by weight of EPA and 35-50 % by weight DHA.

Patentansprüche

1. Verfahren zur Herstellung eines raffinierten Fischölkonzentrates mit einem Gehalt von wenigstens 20 Gew.-% Eicosapentaensäure (EPA) und wenigstens 35 Gew.-% Docosahexaensäure (DHA), wobei die verbleibende Menge an Konzentrat andere ungesättigte Konzentrates vorwiegend als Alkylester von niederen Alkoholen vorliegen, welches Verfahren die Schritte :

(a) Verestern und/oder Umestern der Fett/Fettsäurefraktion des Fischölproduktes bei Raumtemperatur mit einem niederen Alkohol in einer alkalischen Umgebung, die Mengen einer Base enthält, die nur dazu ausreichen, die Veresterungs- und/oder Umesterungsreaktion zu katalysieren ;

(b) Erwärmen des entstandenen Alkylesterproduktes mit einem Überschuß an Harnstoff in einem Alkohol auf 55-90 °C.

(c) Kühlen des Produktes aus Schritt (b) auf 0 °C, um Harnstoff-Fettsäurealkylesteraddukt auszufällen und darauf das genannte Addukt abzutrennen, um eine Lösung zu erhalten, die vorwiegend 3-Fettsäureester und einen unverseifbaren Anteil enthält ;

(d) Abtrennen der 3-Fettsäurealkylester und des unverseifbaren Anteils von der aus Schritt (c) verbleibenden Lösung, vorzugsweise durch Extrahieren mit einem Lösungsmittel ;

(e) Entfernen des Lösungsmittels aus der in Schritt (d) erhaltenen Mischung ; und

(f) Kühlen des in Schritt (e) erhaltenen Konzentrates umfaßt, um Cholesterin auszukristallisieren und andere undefinierte unverseifbare Verbindungen auszufällen und dadurch ein raffiniertes Fischörkonzentrat zu erhalten.

2. Verfahren nach Anspruch 1, worin das in Schritt (f) erhaltene Konzentrat weiter behandelt wird, um die Konzentration von EPA und DHA darin zu erhöhen.

3. Verfahren nach Anspruch 1 oder 2, worin in Schritt (b) die Fettsäurealkylester mit Harnstoff bei einer Temperatur von 60-80 °C behandelt werden, wodurch das Harnstoff-Fettsäurealkylester-Addukt vorwiegend keine 3-Fettsäureverbindungen und unverseifbare Verbindungen enthält.

4. Verfahren nach einem der vorhergehenden Ansprüche, worin in Schritt (d) die Trennung durch Extraktion mit Hexan durchgeführt wird.

5. Verfahren nach Anspruch 4, worin vor dem Schritt (e) der Hexanextrakt mit einer verdünnten Säure, vorzugsweise Salzsäure, gereinigt wird.

6. Verfahren nach einem der vorhergehenden Ansprüche, worin Schritt (f) durchgeführt wird, indem zuerst das 3-Fettsäurealkylesterkonzentrat auf eine Temperatur von nicht weniger als —25 °C gekühlt wird, wodurch Cholesterin auskristallisiert, und darauf auf —50 °C gekühlt wird, wodurch der verbleibende Anteil an unverseifbaren Verbindungen ausgefällt wird.

7. Verfahren nach einem der vorhergehenden Ansprüche, worin im Schritt (a) Methanol eingesetzt wird, wodurch die Fettsäureverbindungen des erhaltenen Konzentrates vorwiegend als Methylester vorliegen.

8. Verfahren nach einem der vorhergehenden Ansprüche, worin das in Schritt (f) erhaltene, raffinierte Fischölkonzentrat 20-30 Gew.-% EPA und 35-50 Gew.-% DHA enthält.

Revendications

1. Procédé pour la fabrication d'un concentré d'huile de poisson raffinée contenant au moins 20 % d'acide éicosapentaénoïque (EPA) et au moins 35 % d'acide docosehexaensoïque (DHA) en poids, le reste du concentré comportant d'autres composés insaturés d'acides gras, et les composés d'acides gras du concentré étant principalement présents sous forme d'esters alkyliques d'alcools inférieurs, procédé qui comprend les étapes consistant à :

(a) estérifier et/ou trans-estérifier la fraction corps gras/acides gras de l'huile de poisson obtenue à la température ambiante avec un alcool

inférieur dans un environnement alcalin contenant des quantités d'une base suffisantes seulement pour catalyser la réaction d'estérification et/ou de transestérification ;

(b) chauffer l'ester alkylique obtenu avec un excès d'urée dans un alcanol à une température comprise entre 55 et 90 °C;

(c) refroidir le produit obtenu dans l'étape (b) à 0 °C pour précipiter le produit d'addition urée-ester alkylique d'acides gras et ensuite séparer ce produit d'addition pour laisser une solution contenant principalement des esters de 3 acides gras et une partie insaponifiable ;

(d) séparer de la solution restante à l'issue de l'étape (c) les esters alkyliques de 3 acides gras et la partie insaponifiable, de préférence par extraction avec un solvant ;

(e) enlever tout solvant du mélange obtenu dans l'étape (d) ; et

(f) refroidir le concentré obtenu dans l'étape (e) pour cristalliser le cholestérol et précipiter tout composé insaponifiable indéfini, ce qui permet de laisser un concentré d'huile de poisson raffinée.

2. Procédé selon la revendication 1, dans lequel le concentré obtenu dans l'étape (f) est en outre traité de manière à augmenter la concentration de EPA et de DHA.

3. Procédé selon l'une quelconque des revendications 1 et 2, dans lequel, dans l'étape (b) les esters alkyliques d'acides gras sont traités avec de l'urée à une température comprise entre 60 et 80 °C, d'où il résulte que le produit d'addition urée-esters alkyliques d'acides gras dans la partie principale ne contient pas des composés des 3 acides gras et des composés insaponifiables.

4. Procédé selon l'une quelconque des revendications précédentes dans lequel, dans l'étape (d) la séparation est effectuée par extraction avec de l'hexane.

5. Procédé selon la revendication 4, dans lequel avant l'étape (e) l'extrait par l'hexane est purifié avec un acide dilué, de préférence avec de l'acide chlorhydrique.

6. Procédé selon l'une quelconque des revendications précédentes, dans lequel l'étape (f) est effectuée en refroidissant d'abord le concentré d'esters alkyliques de 3 acides gras, à une température non inférieure à —25 °C, d'où il résulte que le cholestérol est cristallisé, et ensuite à —50 °C, d'où il résulte que la partie restante des composés insaponifiables précipite.

7. Procédé selon l'une quelconque des revendications précédentes, dans lequel on utilise du méthanol dans l'étape (a), d'où il résulte que les composés d'acides gras du concentré obtenu sont principalement présents sous forme d'esters méthyliques.

8. Procédé selon l'une quelconque des revendications précédentes, dans lequel le concentré d'huile de poisson raffinée obtenu dans l'étape (f) contient de 20 à 30 % de EPA et de 35 à 50 % de DHA.

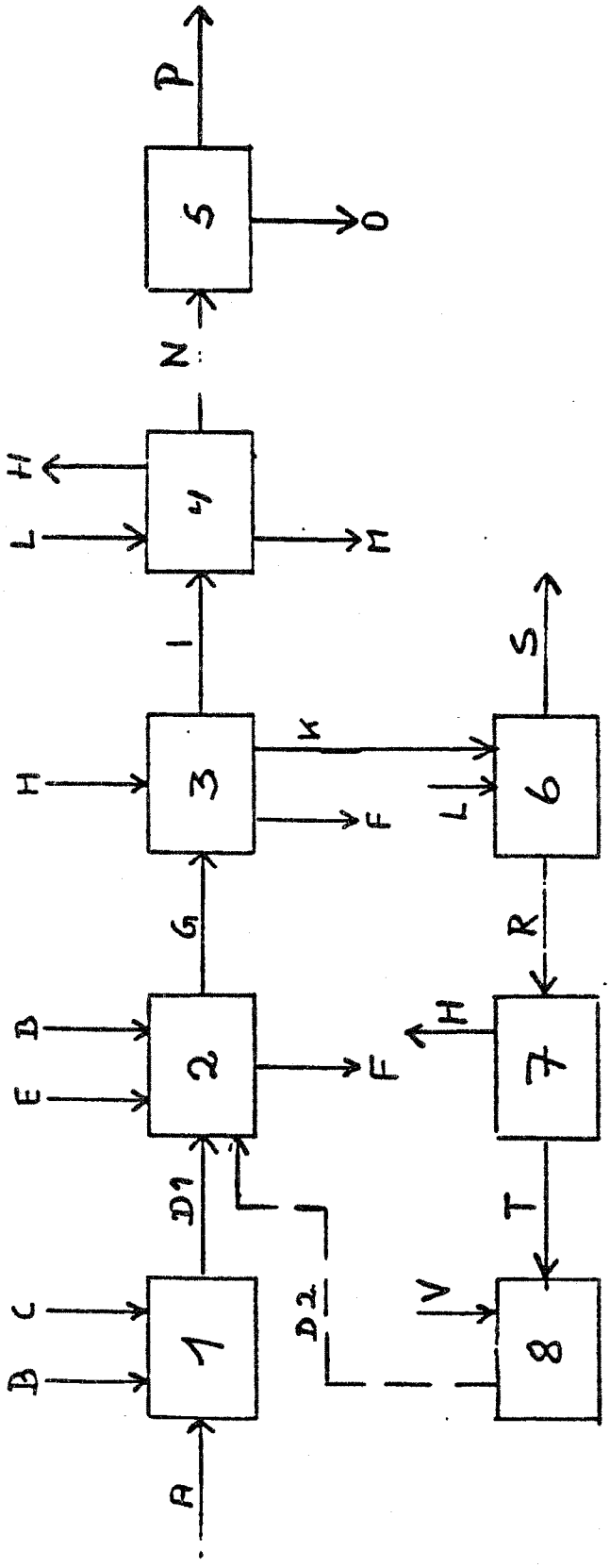

FIG 1